UDC 54.057:547.792

THE SEARCH FOR NEW BIOLOGICALLY ACTIVE SUBSTANCES AMONG DERIVATIVES OF 3-MERCAPTO-4-AMINO-5-CYCLOHEXYL-1,2,4-TRIAZOLE(4*H*)

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Key words: 3-mercapto-1,2,4-triazole; derivatives; alkylation; synthesis; pharmacological activity prognosis

Synthesis of the series of new 4-phenyl-5-cyclohexyl-1,2,4-triazole(4H)-3-yl thioacetanilides is described. The key intermediate – 4-phenyl-5-cyclohexyl-3-mercapto-1,2,4-triazole(4H) has been synthesized started from cyclohexane carboxylic acid through its methyl ester, then hydrazide and the corresponding potassium 3-cyclohexyl dithiocarbazate after cyclisation with hydrazine hydrate. The end products 6a-u have been obtained by alkylation of the key intermediate 5 with chloroacetic acid anilides in the presence of basic catalysts. The purity of the compounds synthesized has been monitored by TLC. The structure of compounds synthesized has been proven by elemental analysis data and NMR spectra. In NMR-spectra the result of alkylation has been proven by disappearence of the chemical shift of the mercaptogroup. All compounds – both intermediate 5 and end products 6a-u contain signals of the cyclohexane system protons as two multiplets near 2.80 ppm (CH) and at 1.92-1.11 ppm (CH_2)$_5$; 4-aminogroup protons as a singlet signal at 5.92-5.87 ppm. The preliminary prediction of the possible pharmacological activity by computer prognosis (PASS programme) has been carried out. Among activities, which are the most probable for some of the substances synthesized, are ligase inhibitor, interferon agonist, antihypertensive, thyroid hormone antagonist, sedative, antiviral (Pa = 0.554-0.729). Due to prognosis and analysis of logical data the substances synthesized will be examined as possible antiviral agents.

ПОШУК НОВИХ БІОЛОГІЧНО АКТИВНИХ РЕЧОВИН У РЯДУ ПОХІДНИХ 3-МЕРКАПТО-4-АМІНО-5-ЦИКЛОГЕКСИЛ-1,2,4-ТРИАЗОЛУ(4Н)
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Ключові слова: *3-меркапто4-аміно-1,2,4-триазол; похідні; алкілування; синтез; прогноз фармакологічної активності*
Описано синтез серії нових 4-феніл-5-циклогексил-1,2,4-триазол(4Н)-3-іл тіоацетанілідів. Ключовий інтермедіат – 4-феніл-5-циклогексил-3- меркапто-1,2,4-триазол(4Н) синтезований, виходячи з циклогексанкарбонової кислоти через її метиловий естер, далі – відповідний гідразид і калій 3-циклогексилдитіокарбазат після циклізації з гідразин гидратом. Цільові продукти були отримані алкілуванням ключового інтермедіату анілідами хлороцтової кислоти в присутності основних каталізаторів. Чистоту синтезованих сполук контролювали за допомогою ТШХ. Структура синтезованих сполук була доведена даними елементного аналізу та ЯМР-спектрів. У ЯМР-спектрах результат алкілування був доведений за зникненням хімічного зсуву меркаптогрупи. Всі сполуки – як інтермедіат, так і цільові продукти містять у спектрах ПМР-сигнали протонів системи циклогексану у вигляді двох мультиплетів біля 2,80 м.ч. (СН) та при 1,92-1,11 м.ч. (CH_2)$_5$ і протонів 4-аміногрупи у вигляді синглетного сигналу при 5,92-5,87 м.д. Проведено попереднє прогнозування можливих видів фармакологічної активності за допомогою комп'ютерного прогнозу (програма PASS). Серед видів дії, які є найбільш ймовірними для деяких з синтезованих речовин, такі як інгібітор лігази, агоніст інтерферону, характерною є антигіпертензивна, антагоніст гормону щитоподібної залози, седативна, противірусна (Pa = 0,554-0,729). Відповідно до прогнозу і логічного аналізу синтезовані речовини будуть вивчатися як потенційні противірусні засоби.

ПОИСК НОВЫХ БИОЛОГИЧЕСКИ АКТИВНЫХ ВЕЩЕСТВ В РЯДУ ПРОИЗВОДНЫХ 3-МЕРКАПТО-4-АМИНО-5-ЦИКЛОГЕКСИЛ-1,2,4-ТРИАЗОЛА(4Н)
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Ключевые слова: *3-меркапто4-амино-1,2,4-триазол; производные; алкилирование; синтез; прогноз фармакологической активности*
Описан синтез серии новых 4-фенил-5-циклогексил-1,2,4-триазол(4Н)-3-ил тиоацетанилидов. Ключевой интермедиат – 4-фенил-5-циклогексил-3-меркапто-1,2,4-триазол(4Н) синтезирован, исходя из циклогексанкарбоновой кислоты через ее метиловый эфир, далее – соответствующий гидразид и калий 3-циклогексилдитиокарбазат после циклизации с гидразин гидратом. Конечные продукты 6а-и были получены алкилированием ключевого интермедиата 5 анилидами хлоруксусной кислоты в присутствии основных катализаторов. Чистоту синтезированных соединений контролировали с помощью ТСХ. Структура синтезированных соединений была доказана данными элементного анализа и ЯМР-спектров. В ЯМР-спектрах результат алкилирования был доказан исчезновением химического сдвига меркаптогруппы. Все соединения – как интермедиат, так и целевые продукты содержат в спектрах ПМР-сигналы протонов системы циклогексана в виде двух мультиплетов около 2,80 м.д. (СН) и при 1,92-1,11 м.д. (CH_2)$_5$ и протонов 4-аминогруппы в виде синглетного сигнала при 5,92-5,87 м.д. Проведено предварительное прогнозирование возможных видов фармакологической активности с помощью компьютерного прогноза (программа PASS). Среди видов действия, которые являются наиболее вероятными для некоторых из синтезированных веществ, таких как ингибитор лигазы, агонист интерферона, характерным является антигипертензивное, антагонист гормона щитовидной железы, седативное, противовирусное (Pa = 0,554-0,729). В соответствии с прогнозом и логическим анализом синтезированные вещества будут изучаться как потенциальные противовирусные средства.

Our previous investigations have shown that derivatives of 3-mercapto-1,2,4-triazole(4H) are very prospective due to their pharmacological activity [1-3]. The next step of our investigation is modification of their structure in order to know how this process will affect the biological action. Analysis of scientific literature for the last 5 years has shown that the presence of aminogroup in 4 position of the heterocyclic ring might be very interesting. Similar compounds with different substituents in 3 and 5 positions were synthesized before and it was found that they had different kinds of the pharmacological action. Thus, for 4-ilydene derivatives the analgesic [4], antiproliferative [5], antianxiety, antidepressant [6], antioxidant [7, 8] action was found, thiosemicarbazone derivatives were active as anti-infective and anticonvulsant agents [9], acylated compounds were investigated for the anti-inflammatory and antinociceptive activity [10]. A great number of the compounds synthesized in these groups showed a significant antimicrobial activity [11-14].

Continuing our previous investigations we have planned to synthesize compounds, which contain both amino- and mercaptogroup in their structure. But we take into account that these groups are rather hydrophilic at the same time; and this fact may be the cause that such compounds can not easily penetrate into the organism, especially through the hematoencephalic barrier. So, we have planned to block the hydrophilic mercaptogroup by its alkylation and introduce the lipophilic cyclohexyl substituent to 5 position (1).

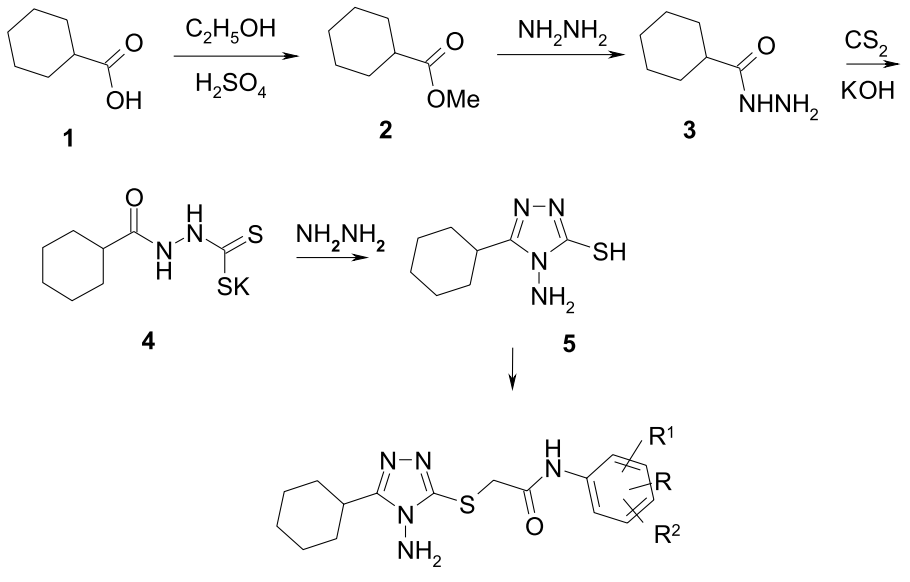
Formation of the basic 3-mercapto-4-amino-1,2,4-triazole(4H) structure in theory is possible in two ways. The first of them is through interaction of carboxylic acid with thiocarbohydrazide under reflux for 4 h [15]. Another one is performed by preliminary synthesis of carboxylic acid hydrazide, its interaction with carbon disulfide and cyclization of sodium dithiocarbazinate into the end product [16]. The last one has been used for solving of our tasks and this way is described in Scheme. As the initial compound methyl cyclohexanoate **2** has been used. Cycloxexanoic acid hydrazide **3** has been synthesized by hydrazinolysis with an excess of hydrazine hydrate. The reaction of carbon disulfide in the alkaline medium leads to formation of the correspondent potassium dithiocarbazinate **4**, which is further converted by the action of hydrazine hydrate into the key intermediate – 3-mercapto-4-amino-5-cyclohexyl-1,2,4-triazole(4H) **5**.

The end products **6a-u** (Table 1) have been obtained by alkylation of thiol **5** with chloroacetic acid anilides in the presence of basic catalysts.

The structure of the substances synthesized has been proven by elemental analysis and NMR spectra data (Table 2).

All compounds – both intermediate **5** and end products **6a-u** contain signals of the cyclohexane system protons as a multiplet at 1.92-1.11 ppm; 4-aminogroup protons are as a singlet signal at 5.87-5.92 ppm. In NMR-spectra the result of alkylation has been proven by disappearence of the chemical shift of the mercaptogroup. For intermediate **5** this signal is a singlet at 13.24 ppm (see *Experimental part*). Instead of it the signals of substituents appear. Besides all NMR-spectra of compounds **6a-u** contain some other general signals. NH-Protons of the amide group are situated as singlets at 9.54-1.70 ppm. We can determine signals of aromatic protons of the phenyl ring due to their intensity and multipleticity in relation to substituents (Table 2).

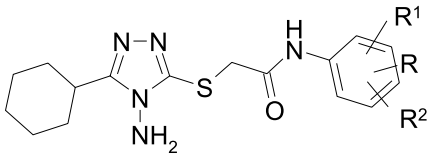
To optimize of the pharmacological screening we used preliminary prediction of a possible pharmaco-



Scheme

Table 1

Yields, melting points for the substances synthesized with the general formula



		R	R^1	R^2	Yield,%	M.p., °C
11180	6a	H	H	H	89	220-2
11206	6b	H	H	2-Et	81	156-8
12682	6c	H	H	4-Et	90	155-7
12887	6d	H	H	4-Pr-iso	78	191-3
11185	6e	H	H	3-F	84	195-7
11209	6f	H	H	3-Cl	83	149-51
11203	6g	H	H	4-Cl	89	194-6
13276	6h	H	H	2-CF_3	81	166-8
11204	6i	H	H	3-CF_3	85	188-90
11197	6j	H	H	2-COOEt	77	102-4
12746	6k	H	H	4-COOMe	78	136-8
12614	6l	H	H	4-COMe	82	184-6
12708	6m	H	H	4-OEt	82	170-2
12679	6n	H	2-Me	5-Me	79	172-4
12764	6o	H	2-Me	6-Me	86	214-6
11189	6p	H	2-Me	5-Cl	77	171-3
11194	6q	H	3-Me	5-Me	84	174-6
12707	6r	H	2-OMe	4-OMe	85	162-4
11199	6s	H	3-OMe	4-OMe	85	159-61
12518	6t	H	2-Cl	6-Cl	87	188-90
11186	6u	2-Me	4-Me	6-Me	87	200-2

logical activity by computer prognosis (PASS programme) [17]. In many cases this programme permitted to conduct pharmacological investigations in a reduced experiment with good results.

Among activities, which are the most probable for the substances synthesized, are ligase inhibitor, interferon agonist, antihypertensive, thyroid hormone antagonist, sedative, antiviral (Pa = 0.554-0.729). It should be noted that the computer programme does not provide a high probability for some of the compounds synthesized probably due to the lack of information on the their structural fragments.

Due to prognosis and analysis of logical data the substances synthesized will be examined as possible antiviral agents.

Experimental Part

Melting points were determined by the open capillary tube. NMR ^1H spectra were recorded on a Bruker WM spectrometer (300 MHz); solvents – $CDCl_3$ or DMSO-d_6; chemical shifts were in ppm, TMS was used as an internal standard. The purity of the compounds synthesized has been monitored by TLC.

Methyl cyclohexanecarboxylate (**2**). To cyclohexanecarboxylic acid (0.1 mol) in methanol (100 mL) add conc. sulfuric acid (5.7 mL) in a round bottom flask. Reflux the mixture for 4-6 h. Distil off an excess of methanol and after cooling transfer the content to a separating funnel containing 100 mL of distilled water. Extract the ester synthesized several times with chloroform (30 mL). Wash the combined organic layers with 20% solution of sodium bicarbonate. After washing with distilled water dry the organic layer over anhydrous $MgSO_4$. Then distil chloroform off under reduced pressure obtaining ester **2.** Yield – 91%, m.p. – 183°C (ethanol).

Cyclohexanecarboxylic acid hydrazide 3. To hydrazine hydrate (99%) (5.7 mL, 0.15 mol) add solution of **2** (0.1 mol) in ethanol in a flat bottom flask dropwise with gentle stirring. After complete addition transfer the mixture into a round bottomed flask and reflux for 4-6 h. Distil off ethanol under reduced pressure. Filter the precipitate of acid hydrazide **3** and recrystallize it from ethanol. Yield – 94%, m.p. – 153°C.

Potassium 3-cyclohexyl dithiocarbazate 4. Treat the mixture of potassium hydroxide (0.15 mol), 100 mL

Table 2

Chemical shifts (δ, ppm) at NMR ^{1}H spectra of the substances synthesized

Comp.	NH, c, 1H	Ar-H	NH_2, 2H, c	S-CH_2, c, 2H	CH, 1H, m	5xCH_2 (cyclohex), 10H, m	Others
6a	10.33	7.54, 2H, d, 7.31, 2H, t, 7.06, 1H, t	5.90	4.08	2.85	1.84-1.11	-
6b	9.74	7.45, 1H, d, 7.23, 3H, m	5.94	4.09	2.81	1.75-1.21	2.73, 2H, m, C$\underline{H}_2$CH$_3$, 1.21, 3H, t, CH_3
6c	10.23	7.46, 2H, d, 7.14, 2H, d	5.90	4.03	2.81	1.81-1.24	2.53, 2H, m, C$\underline{H}_2$CH$_3$, 1.15, 3H, t, CH_3
6d	10.38	7.91, 2H, d, 7.64, 2H, d	5.91	4.08	2.82	1.88-1.23	2.84, 1H, m, C$\underline{H}$(CH$_3$)$_2$; 1.75, 6H, d, C$\underline{H}$(C$\underline{H}_3$)$_2$
6e	10.60	7.61, 1H, d, 7.38, 2H, m, 6.92, 1H, t	5.92	4.09	2.81	1.89-1.25	-
6f	10.54	7.73, 1H, d, 7.34, 2H, m, 6.89, 1H, t	5.91	4.10	2.82	1.90-1.25	-
6g	10.52	7.63, 2H, d, 7.39, 2H, d	5.91	4.09	2.82	1.79-1.30	-
6h	9.99	7.75, 2H, m, 7.46, 2H, m	5.91	4.08	2.73	1.81-1.28	-
6i	10.68	8.07, 1H, s, 7.74, 1H, d, 7.56, 1H, t, 7.42, 1H, d	5.90	4.10	2.85	1.78-1.34	-
6j	10.39	7.47, 1H, d, 7.21, 3H, m,	5.90	4.09	2.81	1.84-1.19	3.94, 2H, m, C$\underline{H}_2$CH$_3$, 1.26, 3H, t, CH_3
6k	10.69	7.93, 2H, d, 7.71, 2H, d	5.90	4.11	2.79	1.88-1.18	3.83, 3H, s, CH_3
6l	10.70	7.96, 2H, d, 7.73, 2H, d	5.92	4.14	2.86	1.81-1.28	2.63, 3H, s, CH_3
6m	10.19	7.45, 2H, d, 6.87, 2H, d	5.90	4.02	2.81	1.81-1.31	3.97, 2H, m, C$\underline{H}_2$CH$_3$, 1.27, 3H, t, CH_3
6n	9.64	7.28, 1H, s, 7.07, 1H, d, 6.89, 1H, d	5.91	4.05	2.83	1.79-1.27	2.25, 3H, s, 2.17, 3H, s, 2xCH_3
6o	9.61	7.07, 3H, m	5.90	4.07	2.75	1.77-1.19	2.07, 6H, s, 2xCH_3
6p	9.73	7.66, 1H, s, 7.24, 1H, d, 7.12, 1H, d	5.91	4.08	2.81	1.88-1.19	2.18, 3H, s, CH_3
6q	10.18	7.18, 2H, s, 6.71, 1H, s	5.90	4.03	2.91	1.79-1.31	2.23, 6H, s, 2xCH_3
6r	9.54	7.79, 1H, d, 6.60, 1H, s, 6.46, 1H, d	5.87	4.01	2.85	1.92-1.30	3.80, 3H, s, 3.74, 3H, s, 2xOCH_3
6s	10.17	7.29, 1H, s, 7.08, 1H, d, 6.91, 1H, d	5.90	4.04	2.86	1.83-1.25	3.75, 6H, s, 2xOCH_3
6t	10.23	7.09, 3H, m	5.90	4.08	2.84	1.84-1.30	-
6u	9.54	6.87, 2H, s	5.92	4.06	2.85	1.88-1.39	2.52, 3H, s, 2.03, 6H, s, 3xCH_3

of absolute ethanol and (0.1 mol) of **3** with (0.15 mol) of carbon disulfide. Dilute the reaction mixture with 75 mL of absolute ethanol and stir at room temperature for 12-16 h. Distil off the solvent under reduced pressure. The salt prepared as described above was obtained in nearly quantitative yield and was employed without further purification.

4-Amino-3-mercapto-5-cyclohexyl-1,2,4-triazole(4*H*) 5. Reflux the suspension of (0.1 mol) of **4** in absolute ethanol, (0.2 mol) of 99% hydrazine hydrate and 6 mL of water for 2-3 h. The colour of the reaction mixture is changed to green with the evolution of hydrogen sulfide gas resulting in a homogenous solution. Add cold distilled water (100 mL) and acidify the solution with conc. HCl. Filter the precipitated solid, wash with 2×30 mL portions of cold water, and recrystallize. Yield – 62%, m.p. – 159-161°C.

NMR spectra: 13.40, 1H, s, SH; 5.48, 2H, s, NH_2; 2.81, 1H, m, CH (cyclohexyl); 1.91-1.32, 10H, m, $(CH_2)_5$, (cyclohexyl).

4-Amino-5-cyclohexyl-1,2,4-triazole(4*H*)-3-ylthioacetanilides (6a-u, Table 1) (general procedure). To the solution of 0.002 mole of 3-mercapto-4-amino-5-cyclohexyl-1,2,4-triazole(*4H*) **5** in 20 ml of ethanol add 20 ml of 0.002 M water solution of KOH. To the solution obtained add the solution of the corresponding chloroacetanilide (0.002 mole) in ethanol with stirring. Reflux the reaction mixture for about 1 h, cool and place it into 200 ml water. Collect and dry the precipitate, recrystallize it from ethanol.

Conclusions

1. Series of new 4-phenyl-5cyclohexyl-3-mercapto-1,2,4-triazole (4*H*) derivatives has been synthesized started from cyclohexane carboxylic acid. The structure of the compounds synthesized has been proven by elemental analysis and NMR-spectra data.

2. Due to prognosis and logical analysis data the substances synthesized will be examined as possible antiviral agents.

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Надійшла до редакції 14.10.2013 р.